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Regulatory relevant and reliable methods and data for determining the environmental fate of manufactured nanomaterials

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Abstract

The widespread use of manufactured nanomaterials (MN) increases the need for describing and predicting their environmental fate and behaviour. A number of recent reviews have adressed the scientfic challenges in disclosing the governing processes for the environmental fate and behaviour of MNs, however there has been less focus on the regulatory adequacy of the data available for MN. The aim of this paper is therefore to review data, testing protocols and guidance papers which describe the environmental fate and behaviour of MN with a focus on their regulatory reliability and relevance. Given the often identified need for modification of OECD testing guidelines, the use of these cannot *per se* be assigned high regulatory

adequacy. Though the specific test considerations will differ between conventional chemicals and MN, the ultimate endpoints of interest are similar. The water compartment must be considered as one of the main points of entry, facilitating dispersion of MN in the environment and establishing a link to the other environmental compartments such as soil, sediment, air, and biota. Once released to water various processes like dissolution, agglomeration, heteroagglomeration, sedimentation, interaction with natural organic matter, transformation and uptake by biota are processes of high relevance for the fate of MN in water. In the review it is found that the OECD draft test guidelines for dissolution and agglomeration will greatly assist in the generation of regulatory relevant and reliable data. Gaps do however exist in test methods for environmental fate, such as methods to estimate heteroagglomeration and the tendency for MNs to transform in the environment.

Keywords:

Engineered Nanoparticles; Degradation; Transformation; Distribution; OECD guidelines

1 Introduction

The production, manufacturing and use of manufactured nanomaterials (MN) in a wide range of products and applications has increased in recent years and environmental release of MN is possible through out the product life-cycle (Nowack et al., 2012; Hartmann et al., 2014). Releases may occur during the use of nano-enabled consumer and industrial products either by intentional (e.g., MN used for environmental remediation) or non-intentional releases (e.g., due to weathering of products containing MN). Environmental emissions of MN may also occur by accidental spills during production or transportation, and when products are disposed of. Therefore, wastewater treatment effluents and sludges, stormwater, landfill leachates and waste incineration residuals are all likely serve as entry points of MN to the environment. Upon emission the fate and behaviour of MN will be determined by their intrinsic properties

as well as the specific environmental conditions (see Figure 1). It is known today that in the environment MN tend to be transformed from their released form (Nowack et al., 2012) and while some analogies can be made to the behaviour of colloids in the environment, the novel physico-chemical characteristics of MN present a challenge in determining their environmental fate and behaviour. A number of recent reviews have adressed the scientfic challenges in disclosing the governing processes for the environmental fate and behaviour of MN (e.g., Peijnenburg et al., 2015; Lowry et al., 2012a) and it is a topic where new experimental insights are published at a rapid pace. While a deeper scientific understanding of underlying processes is crucial to explain MN transformations and distributions in the environment, the urgent need for regulatory decision making regarding the environmental risks of MN calls for reliable and relevant data generated with validated methods (Hartmann et al., 2017). Therefore, the aim of this paper is to review data, testing protocols and guidance papers which describe the environmental fate of MN with a focus on their regulatory reliability and relevance.

Fate processes for MN are predominantly kinetically controlled (Praetorius et al., 2014a), in contrast to the equilibrium based partitioning processes of dissolved chemicals. This means that the regulatory relevance and reliability of environmental fate data for MNs is not easily defined as it is not by default linked to the use of standard or guideline testing, which in most cases are intended to derive equilibrium based partitioning constants.

The regulatory relevance of environmental fate data for conventional chemicals is strongly linked to the use of fate descriptors for the estimation of Predicted Environmental Concentrations (PEC). In chemical safety assessment of industrial chemicals, the software program EUSES (The European Union System for the Evaluation of Substances, https://ec.europa.eu/jrc/en/scientific-tool/european-union-system-evaluation-substances) is applied. This tool contains models for exposure assessment using established chemical fate

models. With regard to MN the final report from the NanoImpactNet FP7 project concluded that the fate models included in EUSES are: "…insufficient for nanoparticles for a number of reasons: (a) the extent/rate of dissolution is unknown or not included in current models and the same goes for (b) the extent/rate of aggregation/settling and (c) the extent of association with sediment." (Hansen et al., 2011). As stated by Peijnenburg et al. (2015) it is known that the traditionally used fate descriptors for chemicals do not apply to nanomaterials and as demonstrated by Praetorius et al. (2012) extensive adjustments of environmental fate models must be implemented before they can be applied to MNs.

The starting point for this review is the fate endpoints addressed in the OECD test guidelines and guidances for conventional chemicals. Though the specific test considerations will differ between conventional chemicals and MNs the ultimate endpoints of interest are similar. The fate endpoints of relevance for MN in the current OECD test guidelines (e.g. OECD TG 105-106, 301--309, 312, 314-317) will automatically be considered of regulatory relevance in the context of this review. With regards to regulatory reliability, papers describing methods with a potential for high reproducibility of methods will be given priority, but it should be recognized that standardized protocols and test guidelines for environmental fate descriptors of MN are currently under development. The use of the test guidelines mentioned above will not *per se* contribute to assigning a high regulatory reliability to the studies reviewed. This review will mainly focus on the fate descriptors for MN in water, and to a less extent on sediments and soils. While the processes described are equally important to the behaviour of MN during ecotoxicological and bioacummulation testing (Hjorth et al., 2017), the focus here is directed towards the fate of MN in the environment.

2 Fate and behaviour of Manufactured Nanomaterials in Water

Surface water may be considered as one of the main points of entry, facilitating dispersion of MNs in the environment and establishing a link to the other part of the environment such as soil, sediment, and biota. As such the aquatic phase may be used as a central starting point to increase the understanding of the environmental fate and behaviour of MNs (Peijnenburg et al., 2015).

Once introduced to the aquatic environment the behaviour of MNs is dependent on the physicochemical characteristics of the MN in question, as well as on the characteristics of the receiving waters (see Figure 1). MNs in natural waters tend to be transformed from the form in which they were released by agglomeration, dissolution, and association with dissolved chemical species and colloidal/particulate matter already present in the natural waters (Nowack et al., 2012; Lowry et al., 2012a; von der Kammer et al., 2012, Peijnenburg et al., 2015). Even though analytical detection of MN in water is theoretically possible, analytical chemical techniques face practical limitations due to interactions with natural water constituents, and background concentrations of naturally occurring elements, chemical species and colloids. Under laboratory conditions, combinations of separation (filtration, ultracentrifugation, chromatography) and analytical techniques (microscopy, spectroscopy, and other analytical approaches) do provide usable data. Unfortunately, these combined techniques are not developed to a level where real time (and long term) analysis under natural conditions is possible (von der Kammer, 2012). Furthermore, recent mesocosm testing (Auffan et al., 2014; Tella et al., 2014; Tella et al., 2015) helps to bridge this gap by providing a reliable methodology to obtain quantitative time- and spatially resolved data on the distribution of MNs within a simulated aquatic ecosystem.

As shown in Figure 1 a number of physical, chemical and biological processes are potentially of importance for the environmental fate of MNs; however, it is also clear that for different

materials, different processes will be of importance. In the water compartment photochemical and redox reactions are identified as potentially significant processes for transforming many MNs, depending on their chemical composition (Hartmann et al., 2014). While dissolution is crucial when considering MNs such as Ag and ZnO, this process is not relevant for other MNs such as CNTs and TiO_2 under environmentally realistic conditions. Agglomeration and subsequent sedimentation, deposition to surfaces, and interaction with NOM should be considered as processes of very high relevance for most MNs in water (see review by Hartmann et al., 2014).

The possible transformations of MNs in soils and sediments are described to a much lesser extent than the transformation of MNs in water. The agglomeration behaviour of most MNs in the water phase can lead to sedimentation of particles (Quik et al., 2011; Quik et al., 2014). In sediments, as well as in soils, MNs are expected to adhere to solids and the available solid surfaces. In the environment redox phenomena occur at a very local scale and can differ with in a few centimeters or even milllimeters. Furthermore, differences in NOM contents may result in very different transformation patterns of MNs between compartments. For quantification of the fate of MNs in the soil and sediment compartments, NOM interaction and redox conditions must therefore be taken into account. Based on the case studies carried out by Hartmann et al. (2014), Figure 2 shows the relative importance of inclusion of the different fate processes in environmental fate modelling of MN in all environmental compartments. As shown in Figure 2, the fate of nano-Ag, ZnO and CuO will be highly influenced by dissolution. For all MN both agglomeration, sedimentation and sorption behaviour are evaluated to be of high importance (see section 2.3 for a definition of the term "sorption" for MN). For all materials biomodification may play a role in their environmental fate, depending on the coating/functionalisation of the MN. Only for CNTs biodegradation may need to be considered, though CNTs should be considered as inorganic MNs as the other

materials in Figure 2. It should however be noted that for organically coated MNs (also those with an inorganic core) biodegradation and biomodification (i.e. biologically induced alterations of MN that are not regarded as degradation) may play a much larger role in their environmental fate than indicated in Figure 2.

In the following the most important of the fate Fprocesses shown in Figure 2 will briefly be described and the current state of availability of regulatory relevant and reliable data and methods reviewed.

2.1 Dissolution

In the REACH implementation project on MN (the so-call RIP-oN) it was stated that "currently available standard methods for measuring dissolution may not be applicable" (Hankin et al., 2011). This was done with reference to OECD TG 105 on Water Solubility (OECD, 1995) and the 2009 preliminary review of OECD guidelines (ENV/JM/MONO(2009)21) (OECD, 2009). As described in the OECD Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media (ENV/JM/MONO(2001)9) (OECD, 2001), dissolution of metals and metal compounds in water may be measured by adding the metal compound to synthetic freshwater followed by agitation, solid-liquid phase separation (by filtration or ultracentrifugation) and analysis for total dissolved metal concentration in the water phase. This guidance is currently recommended for determining the water solubility of metals and sparingly soluble metal compounds in aqueous media under REACH, and specific recommendations for MN are described in an appendix to the ECHA Guidance on Information Requirements and Chemical Safety Assessment (ECHA, 2012a-c). Initiated by WPMN a draft OECD TG for dissolution of MN in aqueous media (OECD, 2017a) is currently under development. This draft OECD TG will outline specific guidance how to carry out measurements for quantifying the dissolution (rate) of NMs in the aquatic environment. As such it is very helpful and of high

regulatory relevance and the reliability of the data generated is expected to increase when tests are carried out according to the test guideline.

The recent paper by Hendren et al. (2015) makes the point that simple assays are sufficient to measure the dissolution rate for systems of interest in order to predict the fate of the MN more specifically and to rank/group various tested MNs. As is the case with the OECD TG draft, Hendren et al. (2015) makes a clear distinction between the dissolution rate (i.e. the delivery rate of metal cations under dynamic conditions) and solubility (equilibrium measurement without rate information). The solubility product for MN may be different from that of the bulk materials as described by theoretical and experimental results (Auffan et al., 2009). From a thermodynamic point of view, K_b (crystal solubility) is assumed to be constant and is routinely approximated using the solubility product, K_{sp}. Regarding nanoparticles, K_b is not solely related to K_{sp} but also to particle size and surface tension (Fan et al., 2006). Some studies have concluded that surface dependent solubility could not be quantitatively predicted by classical thermodynamics (Bian et al., 2011), whereas others have shown the linear size-dependency (TEM-derived particle size) of the solubility for AgNP from 40 to 6 nm, as predicted by the modified Kelvin equation (which implies that surface tension was constant for that size range) (Ma et al., 2012). Furthermore, MN will often have coatings to stabilise against agglomeration and keep the particles suspended (Levard et al., 2012). These coatings can have a huge influence on the dissolution behaviour of MN (Misra et al., 2012).

Under most environmental conditions MN will be present at low concentrations and as MN concentrations decrease to very low levels, thermodynamic laws will predict an increase in the soluble fraction of MN components Hendren et al., 2015; Hartmann et al., 2014). The current draft OECD TG provide specific advice on how to measure the dissolution rate and total dissolved material. This is well aligned with the current literature on the topic, though

the choice of ultracentrifugation as the sole method for phase separation may have to be revisisted. In this respect, the option for inclusion of ultrafiltration is worth taking into consideration.

The dissolution rate of MN depends on the composition of the media in which dissolution takes place (ionic strength, ligands, pH, and temperature) and is highly dependent on MN properties (Nowack et al., 2012). For example, the potential size-dependent solubility of nano-sized particles has been highlighted by a number of papers (e.g., Misra et al., 2012). In the ECHA technical guidance it is specified that "… it is necessary to take into account that water solubility has the potential to increase for materials in the nano-size range" (ECHA, 2012a). However, problems of distinguishing between truly dissolved and dispersed MNs have been highlighted as major chemical-analytical concerns. The methods traditionally applied for phase separation may require specific considerations for MN. Historically the dissolved fraction has been operationally defined by its ability to pass through a filter of a specific pore size, e.g. 0.45 μm (Nowack & Bucheli, 2007; USEPA, 2003). This definition is not applicable when dealing with MN and filtration methods mostly applied for MNs include centrifugal filtration through 1-2 nm membranes (Odzak et al., 2015). Methods to determine dissolution rates of nanoparticles in aqueous media were reviewed by Misra et al (2012) and also included in the current OECD TG draft (OECD, 2017a).

Particle size has been demonstrated to affect solubility and dissolution rate of metal and metal oxide nanoparticles. However, although most of the presently available models agree that the dissolution rate increases with decreasing particle diameter, the relationship is not straight-forward or necessarily easy to demonstrate experimentally. Moreover, it is not always easy to compare results from various studies since dissolution rates are rarely normalised per specific surface area.

Even though there is a complex relationship between solubility/dissolution rate and nanomaterial properties, some general conclusions can be drawn from the literature reviewed. In qualitative terms dissolution rates are likely to increase with smaller particle sizes and this should be taken into account in environmental fate modelling of nanomaterial behaviour in the environment. In line with this, Veltman et al. (2010) proposed dynamic testing and modelling of metal uptake and toxicity, as a way forward, to include AgNP dissolution behaviour during toxicity testing. However, until now only a few studies have focussed on this type of dynamic testing. At the same time, it is clear that the influence of agglomeration behaviour and kinetics as well as of the presence of surface coatings on dissolution need to be examined further. In addition to the physical and chemical properties of MN, the characteristics of the surrounding media (with regard to e.g. pH, ionic strength, hardness, redox environment, and organic matter) as well as the presence of organisms highly affect the dissolution rate of nanoparticles (Levard et al., 2012, Bian et al., 2011, Fol et al., 2012, Yoon et al., 2005). The influence of media characteristics can be either direct (e.g. ligand-assisted dissolution) or indirect (by e.g. affecting agglomeration which in turn influences dissolution) (Misra et al., 2012).

Upon release of the metal ions from MN, the ions will interact with the components of the surrounding media. This leads to changes in the speciation of the metals through formation of water soluble complexes as well as precipitates. The specific formation of metal complexes will depend on media constituents, redox conditions, temperature and pH. Geochemical speciation models can be used as an aid to increase the understanding of the complexation processes (Ma et al., 2014; Levard et al, 2013). However, these models require that metal ion concentrations, media chemistry and relevant complexation and solubility constants are known in detail. While the use of speciation models is a common approach for risk assessment of metals in water these approaches are currently limited by the availability of

reliable solubility constants for most MN (principally due to the difficulty of measuring change of surface tension as particle size decreases).

2.2 Agglomeration, (Hetero)Agglomeration, and Deposition

The role of agglomeration in the fate and behaviour of MN in the environment has been highlighted in a range of studies (e.g. Navarro et al., 2008; Baalousha et al., 2008; Quik et al., 2010). Nanoparticle agglomeration can occur after release to the environment from all stages of the lifecycle of the nano-enabled products, e.g. in production, storage and during handling (Nowack et al., 2012). If MNs are not coated or stabilised, these processes will inevitably occur in water as well as in air (Stone et al., 2010, Lowry et al., 2012a). Agglomeration can occur in river water columns as well as in aquifers in porous media (Solovitch et al., 2010). Agglomeration also occurs in test media of eco-/toxicological tests and during the procedures to prepare test suspensions (Cupi et al., 2015; Cupi et al., 2016). Agglomeration in test media depends on the concentration of the MN and its size, chemical composition, surface charge as media composition (e.g. ionic strength and the presence of natural organic matter) and mixing rates (Lowry et al., 2012b).

The OECD has drafted a test guideline to determine the agglomeration behaviour of manufactured MN in different aqueous media (OECD, 2017b). It defines agglomeration as the "Process of contact and adhesion whereby dispersed particles are held together by weak physical interactions ultimately leading to phase separation by the formation of precipitates of larger than colloidal size (agglomerates)". Due to the weak interactions between particles, agglomeration is in principle a reversible process. In contrast to this, aggregates are defined as clusters of particles held together by strong chemical bonds or electrostatic interactions, i.e. covalent or ionic bonds (ISO, 2011). Aggregation is therefore an irreversible process. As stated by Hartmann et al. (2014), MNs in the environment (or in test media) will not exist in

only one form e.g. *either* primary particles *or* agglomerates *or* aggregates, but will almost always occur simultaneously in a combination of different states. In practice it is difficult, if not impossible, to distinguish between agglomerates and aggregates and these two terms are often used interchangeably. In the following discussion we will use the term agglomeration to cover both processes unless the description specifically relates to aggregation. It is hard theoretically to distinguish the two interacting processes and laborious to quantify them analytically. The draft OECD TG may help to close a gap with regard to comparability of studies. It contains some discussion on the parameters and conditions which influence the homoagglomeration and sedimentation, which is helpful. In addition, it contains the determination of settling kinetics by UV-VIS measurements. However, still it should be recognised that agglomeration may result from different combinations of particle properties and environmental conditions. These may not necessarily be covered by the OECD TG to be developed since this focus on increased environmental realism and detailed process description fall outside the scope of an OECD TG.

As a result of the surface charge associated with very small sizes, it is important to remember that MN display an aptitude to form stable colloidal suspensions over very long periods of time. The stability of nanoparticle dispersions depends on the collision frequency between nanoparticles and the sticking efficiency of these collisions. Two stabilisation processes are known which account for both electrostatic and steric interactions (Cao, 2004). For instance, if all the particles have the same electrical charge (either positive or negative), they will repel one another as they approach each another. The system is then regarded to be colloidally stable (Allen & Smith, 2001). The DLVO (Deryaguin, Landau, Verwey and Overbeek) theory assumes that in any stabilised fluid there are two opposing forces: an electrostatic double layer repulsion that prevents agglomeration and the van der Waals force that binds particles together. Those opposing forces define the sticking or attachment efficiency. In addition,

steric stabilisation differs from electrostatic stabilization in the sense that the surfaces of MNs are chemically modified through adsorption or grafting of polymers from natural solutions (most of the time) forming a molecular corona. Such a corona can serve as particle surface solvation and can prevent their agglomeration by steric repulsive forces (Rose, 2015).

The particulate nature of MN and the presence of a surface with different properties than the core are at the heart of complex phenomena in aqueous systems (e.g., Rose, 2015). It is worth remembering that atoms at the surface differ from atoms in the bulk. In the case of crystallised inorganic oxide particles for instance, surfaces can be considered as a zone of thermodynamic non-equilibrium. Surfaces consist of oxygen atoms with a lower coordination number due to the disruption of the crystal periodicity. This disrupted coordination leads to a violation of the electro-neutrality of minerals between anions and cations and therefore to surface charge. In simple systems and when surface charges are high enough, MN can remain in suspension. However molecular, interparticle interactions in natural systems that can decrease repulsive forces can be at the heart of MN homoagglomeration (MNs attach to each other) and heteroagglomeration (attachment to other surfaces) (Praetorius et al., 2014b) as well as of deposition on porous media (soil).

The composition of the environmental media strongly influences the agglomeration behaviour. In natural systems the modification of surface chemistry by the addition of chemical species that favour either ‘bridge’ formation among the particles or ‘chemical patches’ on the surface will decrease the repulsive energy or increase the attractive energy between particles. Qualitative trends predicted by DLVO-type models have been observed in nanoparticle suspensions - that is, that particles tend to aggregate more quickly at higher ionic strengths and/or at pH values near the isoelectric point.

2.2.1 Heteroagglomeration of MN

In natural waters, heteroagglomeration is more likely to occur compared to homoagglomeration as the expected concentration of MN is low – often many orders or magnitude lower than natural colloids (Praetorius et al., 2012; Gottschalk et al., 2009). It has been shown that heteroagglomeration represents the most important mechanism at the heart of MN mobility in rivers (Praetorius et al., 2012; Sani-Kast et al., 2015). However, even if protocols to measure homoagglomeration are well established, heteragglomeration measurement remains a challenge even if a few papers describe indirect protocols (Praetorius et al, 2014b; Labille et al, 2015). Instruments may not be sensitive enough to detect the interaction between MN and particles during the heteragglomeration processes, since this is a function of the size ratio between MN and natural colloids. Current sensitivity levels permit only the evolution of the largest particles forming the heteroaggregates/agglomerates to be followed. It was shown by Labille et al (2015) that at low ratios between MN and suspended particulate matter (SPM), heteragglomeration was driven by the number ratio while at higher MN/SPM ratio the heteragglomeration was driven by surface ratio. However, the published experiment cannot take into account all the seasonal and spatial variability of SPM and colloid compositions, which can differ in terms of surface properties which then affect attachment efficiency.

2.2.2 Agglomeration and interaction with NOM

A large number of studies have described that the presence of NOM (mostly in the form of humic acid) affect agglomeration of nanoparticles (Hartmann et al., 2014; Sani-Kast et al 2016). However, the influence of natural organic matter (NOM) on the agglomeration of MN is complex, since it can both enhance and reduce agglomeration (Arvidsson et al., 2011). More theoretical work detailed the complexity of nanomaterial – NOM interactions and agglomerate formation. Indeed (Loosli et al., 2015), neatly showed, thanks to an insight into

the thermodynamic association properties, that the mixing order between MN and NOM can change the agglomeration process. Addition of alginate as a NOM model substance, to nano-TiO_2 leads to the formation of agglomerates due to alginate bridging. It is worth noting that the OECD TG draft on agglomeration prescribes that the influence of Dissolved Organic Carbon (DOC) on nanoparticle colloidal stability should be investigated. The current draft includes criteria for which kind of DOC to be used and demands for reporting of the DOC composition. However, the definition of DOC obtained after a 0.45 µm filtration can lead to confusion and may not only address the effect of DOC adsorption at the surface of nanoparticles.

Only a limited number of studies have investigated whether factors other than humic acid (HA) have an influence on MN agglomeration behaviour (e.g. Liu et al. (2011); Prathna et al. (2011)), and even fewer have addressed the role of the structure of agglomerates concerning agglomeration (homo- and heteroagglomeration) and sedimentation mechanisms. Homo- and even more heteroagglomerates do exhibit complex 3D structures that are related to their mechanism of formation which controls their mobility. Agglomerates formed in water can be conveniently described using fractal geometry (e.g. Buffle et al., 1998). In a simplistic vision, the fractal dimension can be considered to be a quantitative measure of the more or less compact nature of the agglomerates (Loosli et al, 2015). The fractal dimension of a cluster depends on the mechanism by which the agglomeration process occurs and the attachment efficiency (e.g. Vikesland et al, 2016). At present it may be premature to suggest that quantification of fractal dimension should be included in an OECD TG. However, from a scientific point of view the fractal dimension of agglomerates is of high importance and should be considered as a crucial parameter to accurately predict and model settling velocity in addition to particle size. For instance, Veerapaneni and Wiesner (1996) demonstrated that the increase of fractal dimension leads to increased flow resistance through the agglomerates.

Fractal agglomerates are therefore expected to behave like objects with a smaller size than equivalent spheres. Fractal agglomerates must therefore be considered as permeable. The fractal dimension i.e. the compacity–porous nature of agglomerates will govern their mobility/velocity but also the interaction with NOM and other molecules. Even though it is a crucially important property, fractal dimension is not considered or characterised in the vast majority of studies, thus failing to describe the actual structure, reactivity and formation mode of agglomerates.

2.2.3 Sedimentation

Agglomeration in the water column may also lead to sedimentation. Prediction and modelling of sedimentation remain a difficult task since in a natural system, in addition to nanomaterial interactions with SPM, size, density and fractal dimension of aggregates/agglomerates will control the settling time (e.g. Vikesland et al., 2016). The gravitational settling of nanoparticles in water is a parameter of high importance for the fate of MN (Quik et al., 2010). Sedimentation is potentially the major removal process of MN from the water phase to the sediment phase. There is a clear link between agglomeration and sedimentation since larger particles and agglomerates will tend to settle more rapidly under gravity. Sedimentation may also be affected by so-called gravitational agglomeration, where the slower settling (smaller) particles/agglomerates/aggregates are captured by the more rapidly settling (larger) particles. The gravitational settling of particles can generally be described by Stoke's law, which implies that larger agglomerates and aggregates will settle faster than smaller single particles. This means that agglomeration will be determining for sedimentation of MNs in the aquatic environment. However, when heteroagglomeration takes place between MN and natural colloids, the settling rate of MN may be affected depending on the type of heteroaggregate. It is important to differentiate primary heteroaggregates, where nanoparticles attach to one colloid and the size of the assembly corresponds to that of the colloid, from

secondary heteroaggregates, where more than one colloid is involved in the assembly leading to a larger aggregate size. The sedimentation behaviour of MN in water has been described in several studies, ranging from ultra-pure water over artificial media with and without added NOM to natural freshwater and all the way to seawater (e.g., Keller et al., 2010; Quik et al., 2010; von der Kammer et al., 2010).

2.2.4 Methods to determine agglomeration

A number of methods exist to determine agglomeration of MNs and these are often used in combination. The most appropriate techniques enable the determination of agglomeration processes and kinetics *in situ* without any sample preparation. Static (Small Angle X-ray Scattering, SAXS) and dynamic light scattering (DLS) can be considered as efficient to obtain particle size, fractal dimension time series. However, they are not appropriate to describe agglomeration of polydispersed and/or very dilute systems. Microscopy techniques like electron microscopy (EM) techniques (e.g. scanning EM (SEM), transmission EM (TEM) or scanning transmission EM (STEM)), scanning probe microscopy techniques (e.g. atomic force microscopy (AFM) or scanning tunnelling (STM)), and also centrifugation techniques (e.g. analytical ultracentrifugation, ANUC) are other examples. There are however some disadvantages due to sample preparation (drying and size change due to capillary forces). This has been described well in the literature and for an overview of uses, applicability, pros and cons of various techniques for MN agglomeration quantification and characterisation we refer to Tiede et al. (2008). The OECD draft TG (Agglomeration Behaviour of Manufactured Nanomaterials in Different Aquatic Media) does not directly propose to determine the agglomeration process (OECD, 2017b). The draft TG proposes to determine the proportion of MNs remaining in suspension (supernatant) and the proportion that settled. Centrifugation is used to separate large agglomerates from MNs remaining in suspension and DLS is

recommended for charactersiation of particle size to assess the concentration at the beginning of the test in case information is not available by manufactures or by other techniques.

Measurement of MN zeta potential is an expression of electro-kinetic potential and can be considered an 'indirect' way of measuring MN propensity to agglomeration (Lowry et al., 2016). However, it should be noted that zeta potential measurements alone may not be sufficient to determine suspension stability and should be complemented by the determination of the isoelectric point (pH_{iep}). Additional measurements of the particle size distribution and/or visual observations using other techniques such as e.g. DLS and microscopy help to interpret the data. Moreover, zeta potential cannot resolve agglomeration due to steric effects, hydrophobic interactions, etc. and so remains of little use to accurately describe agglomeration processes.

In addition to the abovementioned analytical techniques a number of simulation models for nanomaterial agglomeration have been developed. Handy et al. (2012) provides a review of the recent developments of user-friendly software that can be used to predict particle behaviour in aquatic media for ecotoxicity testing. More recently Therezien et al., (2014) developed a more complex computer model. It expanded the existing simple homogeneous agglomeration models to account for heterogeneous agglomeration between NPs and the distribution of natural particles already present in environmental waters. This model calculates a range of variables using the concentration, size and fractal dimension of aggregates with one type of MN, only SPM or a mixture of both. The results of modelling show that NP size is related to their potential to persist in the environment, and this effect increases for low attachment efficiency between MN and background particles (SPM) and also for low concentrations. While these model approaches can serve as a starting point for

evaluating agglomeration behaviour they are at present not recommended as stand-alone tools and should be complemented with lab-based experiments.

2.3 Adhesion/deposition of MN to other particles or surfaces

While the sorption process is most often related to molecules that exhibit a specific affinity to particle surface the the use of the term 'sorption' for MN is quite ambiguous. It can refer to pollutant adsorption onto nanomaterial surfaces or the sorption of MN onto the surface of larger particles or other surfaces. In the first case sorption or more accurately, pollutant adsorption onto a nanomaterial surface will control the pollutant mobility but less likely the nanomaterial mobility itself. In that case the role of nanomaterial can be defined as a pollutant carrier. However, in the second case the sorption of MN or more accurately, the adhesion/deposition of nanomaterial onto soil minerals will directly impact the nanomaterial mobility. In this section we deal only with the second mechanism.

For regulatory purposes the OECD guideline for "Adsorption - Desorption Using a Batch Equilibrium Method" (OECD TG 106) was developed to determine the distribution of a conventional chemical between soil and aqueous phases. The concept of this type of batch experiment is to be able to separate free pollutant from sorbed pollutant at the end of the experiment in a separation step. While this is possible for soluble chemicals, it is technically impossible to isolate 'free' nanomaterial from sorbed nanomaterial. Different technical issues can be highlighted. First, soil particles are selected by a 2 mm sieving process. However, soil fractions in the nanometer range can exist in that sieved soil sample, and can be confused with the MN to be tested. Then, $CaCl_2$ 0.01 M is used in the TG. Such high $CaCl_2$ concentration, even if relevant for pore water, may lead to nanomaterial homoagglomeration. If such a process occurs, then the batch experiment will fail as it cannot distinguish soil particles from nanomaterial homoagglomerates.

Column experiments may represent a more appropriate alternative. The OECD TG 312 "Leaching in soil column" was designed to determine the possible transfer of chemicals through soil (and to reach the aquifer). However, the test was developed for dissolved chemicals and its applicability to MN is questionable. For instance, the protocol to saturate soil particles with the pollutant cannot be used as it is. As with TG106, a high concentration of $CaCl_2$ is required (0.01M) which may lead to homoagglomeration of the MN. Even if column experiments provide estimates of the attachment efficiency, other parameters may influence the outcome of a column experiment, e.g. surface roughness/texture of soil particle (concave soil particle surface), as well as nanomaterial coating and functionalisation. Clearly a specific column test must be developed to better determine the transfer of nanomaterial into soils. A few research groups have already developed column experiments to decipher MN fate in porous media. The detailed protocols help to determine MN-MN attachment efficiency and MN-collector contact efficiency (Solovitch et al, 2010; Raychoudhury et al, 2012). OECD has initiated artan adaption of TG 312 to MN where these attempts may serve as starting points.

3 Environmental Transformation of Nanomaterials

In addition to dissolution, sulphidation of 'thioloprive' metals (such as Ag and Cu) is one of the most important - and most well described - abiotic transformation processes for MN in the environment. Sulphidation is dependent on redox conditions in the environment and as reviewed by Peijnenburg et al. (2015), not only silver but also other types of nanoparticles (such as ZnO, CuO, and iron oxides) are susceptible to sulphidation. For silver nanoparticles it is quite well-described that abiotic transformation processes may significantly alter their fate and behaviour (Kagi et al., 2011). The study by Kaegi et al. (2013) demonstrated that even coated AgNPs will be sulfidised in the sewer system and that no significant attachment to sewer biofilm occurred. When AgNPs enter sewage treatment plants they will mainly

attach to the biosolids, undergo oxysulphidation and be present in this transformed form in sludges and effluents (Barton et al., 2014; Kagi et al., 2013). The sulphidation changes not only limits the dissolution potential, but also the mobility and bioavailability of the AgNPs. The study by Lowry et al. (2012b) shows an example of how abiotic transformation (i.e., the reaction of silver ions and sulphur under anoxic environmental conditions) may influence the fate and behaviour of MN under environmentally realistic testing conditions. In this study it was demonstrated that 18 months after addition of AgNPs to freshwater mesocosms, the speciation of Ag in sediments was dominated by Ag_2S and Ag complexed with reduced sulphur in organic matter (Ag-sulphhydryl). The study measured Ag, and not AgNPs, uptake, but even though AgNPs were transformed to sulphur-containing species, some of the added Ag was taken up by plants, fish and insects in the mesocosms (Lowry et al., 2012b).Another example of how the fate of sulfidised AgNPs may change unexpectedly in real-world systems is given by Thalmann et al. (2015). Here it was shown that ozone treatment of sewage treatment plant effluents, a unit process included in modern sewage treatment plants to remove organic micropollutants from treated effluents,

resulted in the oxidation of silver sulphide and lead to higher toxicity of Ag in the effluent (Thalmann et al., 2015).

As described in the report from the OECD Expert Meeting on Ecotoxicology and Environmental Fate in Berlin in 2013 (OECD, 2014) MNs are expected to undergo alterations in the environment. The conclusion of this meeting was that the relevant conditions, resulting in the transformation of MNs, should be identified and that new TGs or a separate new GD should be developed. It seems that this advice has not been implemented yet, though specific suggestions were outlined at the OECD meeting (OECD, 2014). These included the decision tree shown in Figure 3 for aging and abiotic transformations of MN. It was found that abiotic physical and oxidative degradation are important parameters for the dispersability of MN in

the environment and that these should be determined in a time-dependent manner. Furthermore, not only aerobic conditions should be included, but also anoxic conditions since for example the fate of AgNPs is strongly dependent on redox conditions (i.e. the presence of dissolved sulphide).

With regard to biological degradability of MN it is important to note that initial tests in the OECD biodegradability classification (OECD TG 301 A-F) are focussed on the total mineralisation of an organic chemical. Therefore, these TG are only applicable to carbon-based MN such as graphene oxides, CNTs and fullerenes (C60) (OECD, 2014). In the open literature only one study using an OECD guideline test for biodegradability has been identified. In the study by Hartmann et al. (2011) the OECD 301F (manometric respirometry) test for ready biodegradability was used to test aged C60 suspensions. The testing method was found to be feasible with the conclusion that the aged suspensions of C60 were not readily biodegradable (Hartmann et al. 2011). However, due to the endpoints in all OECD tests of the 301 series (oxygen consumption, carbon dioxide production, or disappearance of total organic carbon content) test concentrations in the mg/L range are needed to be able to measure these end points above the limit of detection of the mehtods. For MN this gives rise to concerns regarding concentration-dependent agglomeration (Baalousha et al., 2016) which may alter the biodegradability of the original nanomaterial. In this context, it should however, be mentioned that materials like graphene oxides, CNTs and C60 are generally considered not to undergo biological degradation by microorganisms (Stone et al., 2010).

Carbon-based MNs may however be transformed by abiotic processes, like hydroxylation (Hou & Jafvert, 2008). This was shown in the aged C60 tested by Hartmann et al. (2011). Such surface modification through aging may make the nanomaterial more susceptible to biological degradation. In a couple of other studies using non-guideline approaches, carbon-

based MNs have been observed to undergo biological degradation. For instance, fullerol ($C_{60}(OH)_{19-27}$) can be oxidised to CO_2 by white rot basidiomycete fungi (Schreiner et al. 2009). In another study carboxylated SWCNTs were degraded in the presence of horseradish peroxidase and hydrogen peroxide (Allen et al. 2009). In the same study pristine SWCNT was not degraded by this treatment and this difference in degradation pattern was suggested to be due to strong adsorption of horseradish peroxidase to the carboxylated form but not to the pristine form of SWCNT. In a more recent study the mineralisation of ^{14}C labelled MWCNT was studied during a 7-day incubation period with a mixed bacterial culture at 39^{o}C (Zhang et al., 2013). A low degree of mineralisation (2-7%) was observed and although this and the above mentioned studies indicate a potential degradability of carbon-based MNs by biological processes their actual relevance in the context of regulatory testing remain to be established.

It should however be noted that organic surface coatings on MNs may undergo biological degradation (e.g., Kirschling et al. (2011). The degradation of surface coatings and stabilisers will greatly influence the environmental behaviour of MNs. Information on the biodegradability of organic surface coatings may in principle be provided by current OECD TGs for biodegradability since the focus will be on the organic chemical and not on the MN. In this case the high concentrations of organic carbon needed for ready biodegradability tests (OECD TG 301) will however constitute a practical problem and the use of ^{14}C-labelling will most likely be needed to determine the ultimate degradability of organic surface coatings (OECD, 2014).

Furthermore, biomodifications of discharged MNs may occur upon ingestion (or other routes of uptake in higher organisms), where the internal conditions of the organism alter the MN properties (Roberts et al., 2008; Tangaa et al., 2016). Biomodification may also occur when organisms, such as algae, release exudates which bind to the MNs and change their properties

(Hartmann et al., 2013). Although some information is available on biomodifications of MNs, their influence on MN fate and transport is still poorly understood (Kirschling et al, 2011; Montes et al, 2012).

In the case of soils and sediments, microorganisms may also indirectly transform MN: aerobic or anaerobic conditions due to microorganism metabolism may lead to significant changes in redox potentials. As discussed above, some metal-based MN exhibit redox instability leading to either oxidative (e.g. Ag) or reductive dissolution (e.g. CeO_2, Schwabe et al., 2015). The OECD TG 307 "Aerobic and Anaerobic Transformation in soil" was developed to determine the transformation of chemicals in soils. As for many other OECD TGs this test was developed for soluble chemicals and may not be appropriate for MN. One of the most difficult issues to tackle is the almost impossible task of separating the MN from soil particles at the end of experiment. In the specific case of nanomaterial transformation, it could be valuable to implement the test by partly isolating MN from soil particles in order to facilitate recovery of MN by the end of the test. For instance, in studies focussed at the role of dissolved chemical species released from MN, the use of dialysis bags in which MN could avoid complex separation techniques in soil studies and couldassist in mass balance determination before and after experiment.

4 Conclusions and Recommendations

Once in the environment, the fate and behaviour of MN will depend on the physicochemical properties of the MN, the environmental transformation processes and specific environmental conditions. It is well known that upon release to any environmental compartment MN will undergo a series of dynamic transformation processes. However, the complexity of understanding and describing these makes the definition of simple test methods difficult when the focus is on regulatory relevance and reliability. The OECD draft TGs and GDs for

environmental fate endpoints (e.g., dissolution, agglomeration) will all contribute to increase the relevance and reliability of test results.

In the literature review for this paper, surface affinity is identified as a parameter that may provide significant insight into the environmental fate and behaviour of MN. Surface affinity is a crucial parameter in determining the attachment of nanoparticles in environmental matrices and their tendency to heteroaggregate (Therezien et al., 2014). The surface affinity of a given MN can in principle be determined as a function of MN surface properties and the medium used for dispersion or the matrix in which they are embedded (Hendren et al., 2015). Hence it is recommended that test guidance on determination of surface affinity should be considered in the further work of the OECD WPMN. There are currently a number of knowledge gaps with regard to predicting the environmental fate and behaviour of MN. However, as described by Peijnenburg et al. (2015) the distribution between sedimented and suspended forms of MN as well as their transformation by chemical or physical reactions, in combination with any biological transformation are key factors determining the fate of MN in the aquatic environment. This is in accordance with the findings of this literature review based on which the following specific knowledge and methodological gaps were identified:

Dissolution/precipitation/speciation processes

Dissolution kinetics is of high importance for a number of MN such as ZnO, Ag, and CuO. The test guideline under development for dissolution is expected to facilitate the generation of comparable results of high regulatory relevance and reliability. This is urgently needed since only a few studies so far have focussed on dissolution kinetics. Such tests can provide valuable information for a first grouping based on dissolution behaviour. In that sense, this TG can provide reliable results for a better comparison between various types of MN. To address the need for environmental relevance of dissolution data a tiered approach could developed in a OECD GD with dissolution in

water determined according to an OECD TG as an entry level and including a second tier of tests under more complex conditions. However, abiotic mechanisms can be strongly modified due to the presence of living organisms. For instance, bacteria, algae and plants can produce and release a large variety of complexing agents that may be involved in metal-nanoparticle dissolution. Such biological driven mechanisms can modify the dissolution rate significantly. Furthermore, other (abiotic) parameters can influence the dissolution rate such as agglomeration or the presence of natural organic matter. Thus, the role of these influencing or interfering parameters need to be studied further so that solid and quantitative conclusions can be drawn and can be incorporated in an OECD TG for dissolution and/or in an OECD GD providing a tiered approach with increasing complexity and environmental realism similar to what is done today in the OECD testing scheme for biodegradability.

Agglomeration processes

Due to the very high importance of agglomeration for the environmental fate of all types of MN, the development of an OECD test guideline on agglomeration in aquatic media is highly relevant. It may help to close a gap with regard to comparability of studies, but still it should be recognised that agglomeration may result from different combinations of particle properties and environmental conditions. It has been recognised that the proportion of manufactured MN versus natural colloids in fresh water clearly supports heteroagglomeration as the major mechanism. It is therefore important to go beyond adressing homoagglomeration. A higher tier test or guidance for the determination of heteroagglomeration should be developed and standardised. As suggested for dissolution above a tiered approach with increasing complexity and environmental realism similar to what is done today in the OECD testing scheme for biodegradability should be considered. Models have been developed for predicting the aggregate/agglomerate size distribution under different thermal and shear conditions,

but the literature review revealed a lack of studies focused on these kinetic changes for MN in environmentally relevant media. Furthermore, a number of studies show that humic (and fulvic) acids and natural organic matter stabilise various types of MNs, but there are exceptions to this rule. In surface waters sedimentation of MN may play a major role, but the extent to which this occurs for different MN is generally unknown. An OECD TG on agglomeration must prescribe that the influence of Dissolved Organic Carbon (DOC) on nanoparticle colloidal stability should be investigated. This would include criteria for which kind of DOC to be used and demands for reporting of the DOC composition. The definition of DOC obtained after a 0.45 µm filtration must be revisited since this filtration cannot separate colloidal and the truly dissolved fraction of organic carbon. As function of the nanoparticle and organic matter size, adsorption of NPs on NOM or even heteragglomeration can occur, but will not be detected.

Transformation processes

It is evident that MNs undergo alterations in the environment and recommendations of OECD (2014) on identification and inclusion of relevant conditions that result in transformation of MNs in a separate GD should be followed. Biodegradability evaluations are only relevant for carbon-basedMN, but only very few studies have provided data that can be used in a regulatory context. The literature shows evidence of persistence of CNT, C60, and carbon black (Hartmann et al., 2014), but for other types of MN, e.g. hydroxylated fullerenes, biological degradation may play a role in reducing their residence time in the environment. This, however, remains to be studied and data are lacking for tests carried out according to OECD test guidelines. Though tests for ready biodegradability (OECD TG 301 tests) may face practical obstacles for certain MNs (e.g. due to the need for relatively high concentrations of dissolved organic carbon (approximately 20 mg DOC/L)), test for inherent biodegradability

(OECD TG 302 tests) and simulation tests (OECD TG 314) are also lacking. In the latter more realistic test conditions and lower concentrations of test substances are allowed, however the costs of such experiments are also higher than for the screening tests for ready biodegradability. While it is almost certain that bio-modification will take place under environmental conditions, very little is known about the processes themselves or how bio-modification will influence the fate and behaviour (and effects) of MN in the environment.

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Figure captions:

Figure 1 Potential transformations of MN in the environment. The figure illustrates how "as released" MN are transformed by a combination of interlinked processes. The resulting environmental fate and behaviour of the MN is further influenced by the properties of the transformed MN in combination with the environmental conditions in question (modified from Hartmann et al., 2014).

Figure 2 Heatmap showing the relative importance of distribution and transformation processes for environmental fate of MN for selected materials. Color code: The darker the shade, the higher importance the process. Ag: Silver, TiO_2: Titanium dioxide, ZnO: Zinc oxide, CNT: Carbon nanotubes, CuO: Copper oxide, CeO_2: Cerium dioxide). Based on data in Hartmann et al. (2014).

Figure 3 Suggested decision tree for consideration of abiotic transformation processes of MN (OECD, 2014). "t" refers to different time points. ND: Non-dispersible; D: Dispersible; S: Soluble.

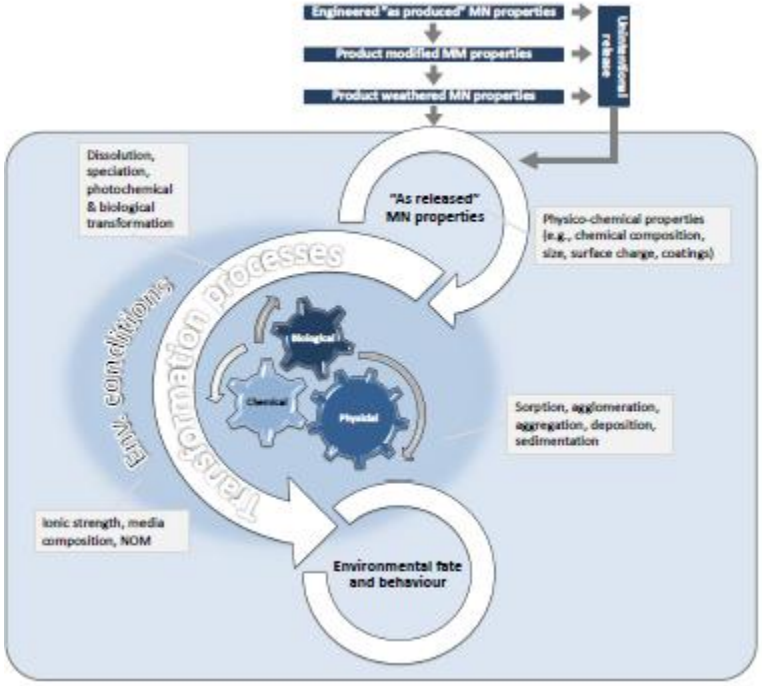


Fig. 1

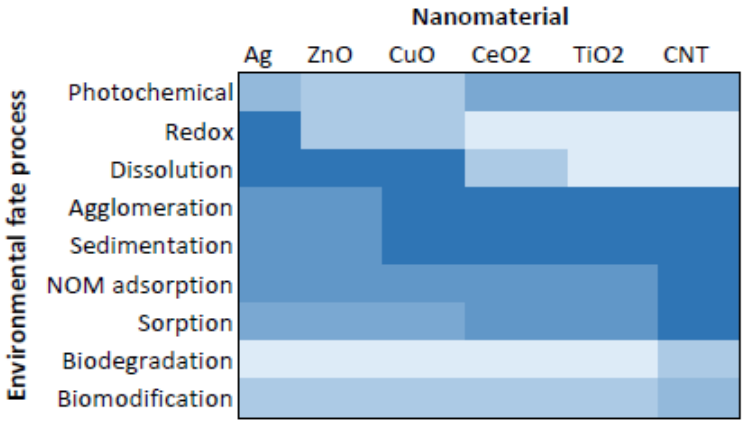


Fig. 2

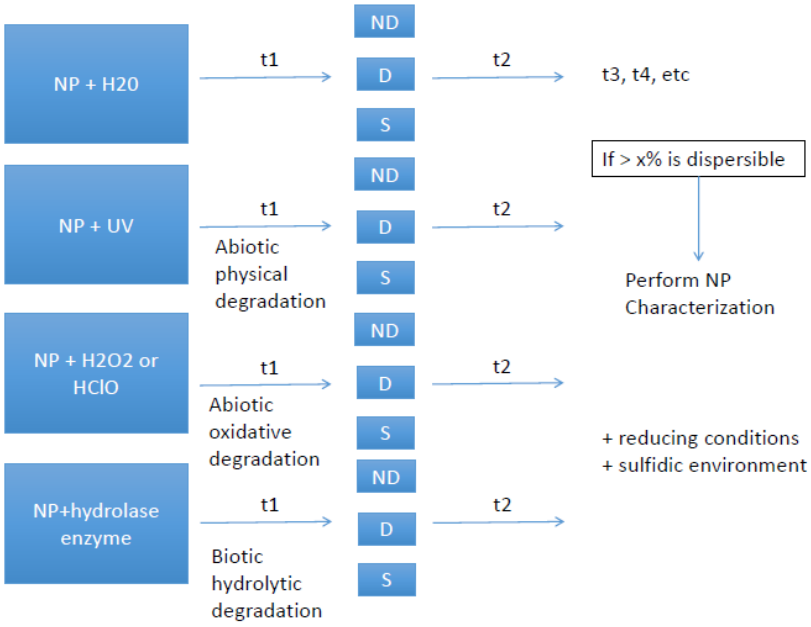
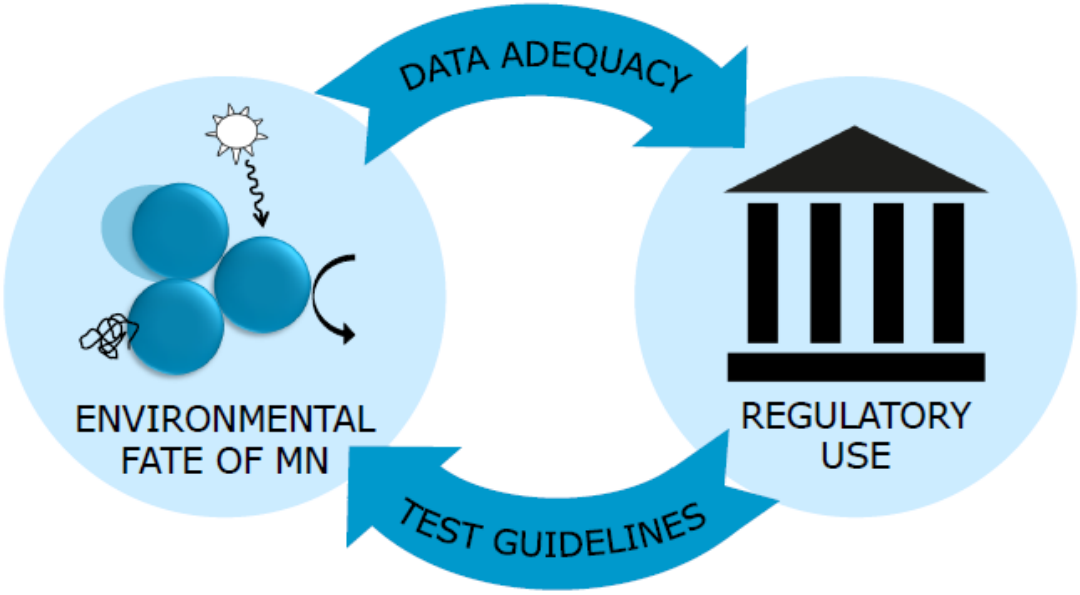


Fig. 3



Graphical abstract

Highlights

- Assays for quantification of heteroagglomeration must be developed to increase the regulatory adequacy of environmental fate data for MN
- Determination of dissolution kinetics for MN is of high regulatory relevance
- Increased focus on transformation processes for MN is needed for environmental fate predictions